The United Nations University
FISHERIES TRAINING PROGRAMME

unuftp.is Final project 2014

PHYSICOCHEMICAL CHANGES IN FROZEN HERRING

Dandan Ren
Dalian Ocean University
Heishijiao Street 52, Dalian, 116023, China
rendandan@hotmail.com

Supervisors:
Dr. Magnea G. Karlsdottir, Matis, Iceland
magneag@matis.is
Prof. Sigurjon Arason, University of Iceland and Matis, Iceland
sigurjon@matis.is

ABSTRACT

The objective of this study was to investigate the effects of different frozen storage temperature (-25°C and fluctuant -12°C) on lipid degradation and protein conformation changes of frozen herring and compare the physicochemical changes of herring frozen at different post-mortem period (frozen on board and frozen on land). Analysis of herring stored at fluctuant -12±3°C for 4 weeks and constant -25°C for two weeks were performed after 0, 2, 4 and 6 weeks of frozen storage. While the stable -25°C samples were analyzed only after 0 and 6 weeks of frozen storage. The effect of the physicochemical parameters were studied by measuring liquid holding capacity (LHC), drip loss, peroxide value(PV), TBARS, free fatty acids (FFA), lipid content, water content, pH value, disulfide bonds content, available and total SH groups. The results showed that lower temperature -25°C can effectively reduce drip loss, especially inhibit the lipid degradation including PV, TBARS and FFA of herring, compare with fluctuant -12°C. Freezing herring during pre-rigor period can slow down lipid oxidation including PV and TBARS, and reduce conformational changes of protein caused by freezing process compare with post-rigor frozen herring.

Keywords: herring, frozen, post-mortem, fluctuant temperature, liquid holding capacity, drip loss, lipid degradation, protein conformation

This paper should be cited as:

Ren, D. 2015. *Physiochemical changes in frozen herring*. United Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/Ren14prf.pdf

TABLE OF CONTENTS

LIST OF FIGURES

1 INTRODUCTION

1.1 Background

Freezing is one of the most common procedures applied to preserve physicochemical properties and prolong storage life of fish, especially for fatty fish because of their high contents of unsaturated fatty acids. The main purpose of freezing is to prevent or slow down bacterial spoilage, enzyme activity and oxidation reactions.

Sensory, chemical and physical changes continue to some extent during frozen storage (Haard, 1992). Undesirable changes associated with lipids and proteins are the main reasons for reduced fish quality during frozen storage.

Quality changes of frozen fish during storage can be influenced by many factors including fish species, the biological status of fish at catch, handling on board, temperature and storage time before freezing, freezing rate, frozen storage temperature, temperature fluctuations, thawing procedure and protection from light and oxygen (Nielsen and Jørgensen, 2004).

Optimal handling and transport conditions can be used to ensure high quality of fish products on the market (Ólafsdóttir, 2005). However, temperature fluctuations through the production and distribution chain could affect the fish quality and safety. These fluctuations mainly occur during handover from one party/function to the next in the logistics.

Sea transportation is a cheap means of transport and the product temperature is generally well-controlled during the whole logistics inside refrigerated containers. Air transportation is quick but expensive and with several hazardous steps during ground and flight operations regarding temperature abuse (Mai, 2010). For frozen fish products, the main transportation method is by sea where the product is kept in refrigerated containers to maintain the required low temperature for the whole voyage. This mode of transportation takes much longer time than by air freight, but the temperature is much more stable.

However, there are still some temperature fluctuations of products in containers. In almost all trips, containers of frozen fish will be placed on the left and right of the ship's center of gravity. Containers of frozen fish are therefore more likely to be directly exposed to the environment than other containers (Keller, 2006). In summer, sunlight is strong and the ambient temperature is high. The heat will be absorbed by the exposed surfaces of the containers causing temperature inside the containers to fluctuate. Moreover, temperature fluctuations in fish within a container may vary depending on the position of the pallet in the container.

China is the largest seafood producer in the world and also a major seafood re-processor of imported seafood. In recent years, China's imports and exports of seafood have seen rapid development. Large majority is the frozen re-export trade. In China, re-processing plants import frozen raw material, thaw and re-process, then export final frozen products to countries such as Japan, USA, Russia and Europe. The plants re-process fish such as Alaska pollock, Pacific cod, Atlantic cod, hake, pink salmon, mackerel, herring, tuna, and also squid and shrimp from all possible origins. Sea transport is the main means of frozen fish transportation for both import and export in the fish trade.

The temperature requirements for frozen fish is at least -18°C, but international and national regulations always recommend -24°C. Strict compliance with temperature must be ensured during transport. Some transport companies set container temperature at -18°C for economic reasons. Shipping between China and Europe takes 30-40 days. The temperature of frozen fish products fluctuates and sometimes goes up to -12°C, even to -8°C, due to the ambient conditions, the position in the container, duration of transport, sometimes even mechanical breakdown or human error (Keller, 2006).

Herring is a popular fatty fish and usually frozen before being processed. Herring frozen on board is usually quickly cooled down to -1.5°C and within a few hours it has been graded, headed, gutted, filleting, packaged, plate frozen and stored at -25°C. The herring is frozen either in rigor or pre--rigor. Herring frozen on land is commonly kept in refrigerated seawater at -1.5°C for 2-3 days before processing and freezing. During that time the herring has gone through rigor mortis and is frozen post-rigor (Love, 1962; Skjervold, et al., 2001b).

So there are some questions when shipping frozen herring: How does fatty frozen fish react to temperature fluctuation? How does the lipid oxidation and protein denaturation affect the physical parameters of the fatty fish during the frozen storage time? Is there any difference between herring frozen on board and frozen on land? Will post-mortem affect the physicochemical changes during frozen storage?

1.2 The Aim

The aim of this project is to explore the influence of time and temperature during frozen storage on protein conformation and lipid deterioration of herring by comparing temperature fluctuation sample with constant temperature sample, to explain the relationship among the liquid holding capacity, drip loss, lipid degradation and protein conformation changes. The result of this study will be used as a reference to provide suggestions to the logistics enterprises in China or other countries which transport frozen fish setting -18°C or even higher temperature.

1.3 Objectives

A deeper knowledge is needed about the influences of handling on board, storage time and storage temperature on the physicochemical changes of frozen fish. The objectives of the study are:

- To study the effects of different frozen storage temperature on lipid degradation and protein conformational changes of frozen herring.
- To compare the physicochemical changes of fish muscle under different handling methods (frozen on board and frozen on land).

2 REVIEW OF LITERATURE

2.1 Atlantic herring (*Clupea harengus*)

Herring is a pelagic fish and found on both sides of the north Atlantic. In the northeast Atlantic it occurs from the Bay of Biscay in the south to Spitzbergen and Novaya Zemlya in the north, while in the northwest Atlantic it occurs from the coast of Maine northwards (FAO, 2014). Herring is not normally gutted at sea, because it is impractical to handle large numbers of small fish on board in a short time. Chilling or freezing soon after capture is therefore all the more important to prevent spoilage (FAO, 2014).

The chemical composition of herring varies considerably with season and maturity. The fat content may be less than 1% immediately after spawning, and more than 20% as spawning time approaches again. The water content of herring decreases as the fat content increases. There are abundant fish lipids in herring, especially polyunsaturated omega-3 fatty acids which have been reported to have beneficial health effects to consumers. Herring as a food has a high energy value because most of the fat is in the flesh (FAO, 2014).

2.2 Temperature in cold chain

In order to ensure product quality and stability, controlled temperatures throughout the cold chain is necessary. Therefore, the required temperature must be maintained from production to consumption. The international and national regulations about the storage temperature of frozen foods set -18°C as the highest temperature during storage and distribution. However, there are some weak links or interfaces in the cold chain that is hard to maintain stable temperature, such as delivery, unloading operations, loading and temporary storage where pallets are generally handled in an uncontrolled ambient temperature (Moureh and Derens, 2000).

Fluctuating temperature accelerates the growth of specific spoilage organisms as well as pathogens (Rediers *et al*., 2009), which can cause quality and safety problems and economic losses. The extracellular formation of ice crystals is accelerated during temperature fluctuations and cellular disruption is increased (Hagyard *et al*., 1993; Bak *et al*., 1999). Temperature fluctuations will cause formation of large ice crystals which have negative effects. Since large ice crystals will cause the bad effect to products, it is important to consider the effects of temperature fluctuations, especially during distribution and retailing. The effects fluctuations bring to the product are depending on storage temperature. Gormley *et al* (2002) studied the quality changes of frozen food at three temperature fluctuation cycles of -30°C to -10°C to -30°C on consecutive weeks or at a constant -30°C for 8 months. The results showed that the temperature regimes had a large effect on lipid degradation products including peroxide (PV) and free fatty acid (FFA) values. PVs and FFAs increased with the frozen storage time.

For the pallet in logistics, the position and surface of product also affect fluctuation of temperature. The temperature rise is totally different between a product placed at the top and another product placed in the center of the pallet. The top layer is very sensitive to ambient conditions and experiences larger fluctuations, whereas in the center, the temperature remains relatively constant (Rediers *et al*., 2009; Raab, *et al*., 2008). It had been found that products or boxes which have more free surfaces to the surroundings, such as at the top corners of a pallet, are more sensitive

to environmental changes (Moureh and Derens, 2000). Nowadays, some methods are studied in order to decrease the negative effects of ambient temperature fluctuations on product temperature (Moureh *et al*., 2002).

2.3 Protein changes in frozen fish

Although frozen storage is an excellent method of preservation, the quality of fish is still decreased and some chemical and physical changes of protein occur such as the changes of muscle texture and flavor loss which is liable to cause consumer rejection (Mai, 2010). Protein denaturation during frozen storage will lead to decreased protein solubility and water holding capacity and to increased drip loss (Piggott and Tucker, 1990).

The mechanism of changes in fish muscle proteins causing insolubility and formation of aggregates during frozen storage is mainly related to the formation of ice crystals, rate of freezing, presence of formaldehyde and lipid hydrolysis and/or oxidation (Saeed and Howell, 2002; Badii and Howell, 2002b; Karlsdottir *et al*., 2014).

During frozen storage, the formation and accretion of ice crystals can result in dehydration, increase in salt concentration and pH changes following the removal of water through ice formation (LeBlanc *et al*., 1988). Lipid hydrolysis and/or oxidation as well as the formation of formaldehyde (FA) by trimethylamine N-oxide dimethylamine also can induce denaturation of proteins. FA acts as a cross-linking agent among proteins and it can toughen texture during frozen storage (Ben-gigirey *et al*., 1999). The susceptibility of fish species to changes induced by frozen storage is significantly different (Badii and Howell, 2002a). During frozen storage, the protein of fatty fish undergoes denaturation or denaturation-aggregation, which bring about significant deterioration of the texture of fish (Sikorski *et al*., 1976). These changes, which are attributed largely to alterations in the myofibrillar proteins, depend not only on species, but also on technological factors such as processing prior to freezing, or storage conditions including storage temperature, temperature fluctuation and storage time. (Haard, 1992; Mackie, 1993; Careche *et al*., 1998).

Protein denaturation is associated with the formation of disulfide bonds and conformational changes in proteins such as changes in secondary and tertiary structure. And it will increase the aggregation of β-sheet structure and decrease in α-helical structure of proteins (Carton *et al*., 2009). The conformation of protein is stable at specific environmental conditions such as temperature, pH and pressure. The structural instability may occur due to the changes of pH, temperature and the presence of other denaturing agents such as salt. All these changes may lead to alternative protein conformations.

The myofibrillar proteins play an important role in water retention of the fish muscle. The amount of water retention depends on the amino acid composition and conformation of the protein. The sulfhydryl groups are regarded as potent crosslinking agents in proteins, and are more easily reactable and accessible for chemical determination in fish myosins (Sikorski *et al*., 1976). Mao and Sterling (1970) found that freezing followed by immediate thawing (freezing/thawing), as well as frozen storage for 30 days at -5°C, of Sacramento blackfish reduced the content of readily reactive -SH groups in extractable myosin by 7 and 9%, respectively. The disulfide bonds can be formed through sulfhydryl oxidation and disulfide exchanges (Visschers and Jongh, 2005; Zhou

et al., 2014). Therefore, sulfhydryl groups and disulfide bonds are always measured to reflect the conformation changes of fish protein.

2.4 Lipid changes in frozen fish

Lipids play roles as a structural component of the muscle membranes, storage droplets of triacylglycerol between muscle fibres and adipose tissue. Depending on the lipid content, fish is classified in three different groups: lean fish in which lipid content is less than 2%, semi-fatty fish in which lipid content is about 2-10%, and fatty fish in which lipid content is more than 10%.

Changes in lipid occur in fish muscle through hydrolysis and/or oxidation mechanisms. Lipid oxidation in fish muscle, especially in fatty fish and semi-fatty fish, is one of the major deteriorative reactions causing a loss in quality during storage. Lipid oxidation negatively affects the color, odour and flavour, functionality and conformation of protein and nutritional content of fish muscle. Lipid oxidation has been shown that it can induce cross-linking of muscle proteins and pigment proteins, resulting in lower whiteness value and decrease gel formation capacity of fish muscle (Lauritzsen *et al*., 1999).

It has been reported that there might be a high risk of rancidity during prolonged frozen storage due to the fatty nature of fish (Sohn and Ohshima, 2010). But fatty fish is attracting a great attention due to the positive effects of marine lipids on human health (Aubourg *et al*., 2007). There is an increasing interest in commercializing it in the frozen state because low temperature can prolong the storage life of fatty fish (Undeland and Lingnert, 1999; Lehmann and Aubourg, 2008), although its storage life is relatively short because of enzymatic and non-enzymatic rancidity development of the highly unsaturated lipid composition (Stodolnik *et al*., 2005).

The storage life of fatty fish is primarily limited by the rancid flavours that are produced through lipid oxidation which can also be linked to proteins deterioration during frozen storage (Sikorski *et al*., 1976). In frozen fatty fish, oxidative changes in lipids (Saeed and Howell, 1999) and pigments affect the odour and color as well as proteins (Saeed and Howell, 1999; Saeed and Howell, 2001).

During frozen storage, lipid hydrolysis can occur enzymatically in fish muscle (Shenouda, 1980). The free fatty acids (FFA) is formed from lipids by enzymatic reactions. Enzymatic reactions can still continue in frozen fish even at -30°C. Lipase activity is the principal cause of hydrolysis and formation of FFAs during frozen storage. The formation of FFA by hydrolysis of muscle lipids is a factor implicated in protein denaturation during frozen storage (Paredi *et al*., 2006).

Boran *et al* (2006) reported that FFA is a measure of hydrolytic rancidity, the extent of lipid hydrolysis by lipase action. Examining the extent of lipid hydrolysis was considered important because of the important lipid hydrolysis development during frozen storage and also because of the great incidence of free fatty acids on lipid oxidation (Stodolnik *et al*., 2005). Among the different biochemical indices checked, FFA has shown to be the most reliable to assess the quality loss in both treated and untreated fish because a good correlation value was obtained with time and with sensory attributes.

2.5 Rigor mortis

Rigor mortis is a process that takes place post-mortem (Kiessling *et al*., 2006). This transformation is critical for the quality of the end product and is directly affected by both pre- and post-mortem factors (Stien *et al*., 2006). The reason for rigor mortis is that after death of the fish, oxygen in the muscle decreases, resulting in lack of energy. This in turn leads to a strong contraction of the muscle fibers (actin-myosin).

The onset and development of rigor mortis depends on the fish species, temperature and handling before catching, pre-slaughter stress, the biological status of the fish and temperature of pre-rigor storage, and shows large individual variation (Azam *et al*., 1989; Jerrett *et al*., 1998; Skjervold *et al*., 2001a). The stress prior to slaughtering and high storage temperature result in faster and stronger rigor contraction (Skjervold *et al*., 2001a).

The rigor process consists of an initial contractile phase (Tornberg *et al*., 2000), during which the muscle fibers contract, and a second stiff phase that a permanent binding of the contractile proteins myosin and actin happens. This process can be influenced by post-mortem temperature. In wild-caught fish, in which pre-slaughter stress and temperature at death are difficult to control, rigor take place within short time after catching, such as herring goes into rigor mortis on average within 2 h after catching (Hattula *et al*., 1995). Storage temperature is accepted as being a major factor affecting rate of quality loss and storage life (Dawood *et al*., 1986). So for wild-caught fish, reducing the fish temperature after catching as soon as possible is an effective way to delay the onset of rigor mortis and also prolong the rigor process. Sea-frozen fillets are generally frozen soon after catch (Martinsdóttir and Magnússon, 2001).

3 MATERIALS AND METHODS

3.1 Experimental design

Commercially available frozen herring (frozen on land and frozen on board) were used in this study. The Atlantic herring (Clupea harengus) were from Síldarvinnslan (www.svn.is) and Samherji (www.samherji.is), which were caught in November 2014. Herring was filleted before freezing and block frozen. Upon arrival to the laboratory, each frozen fish blocks were divided into equal sized pieces, packed into plastics bags and stored at -12±3°C and -25°C for 6 weeks (Figure 1). Experimental analyses of samples stored at fluctuant conditions, -12±3°C for 4 weeks and subsequent constant conditions, -25°C for two weeks were performed after 0, 2, 4 and 6 weeks of frozen storage. While the stable -25°C samples were analyzed only after 0 and 6 weeks of frozen storage. The different combinations of frozen storage time, frozen storage temperature and frozen time after catching were discussed in this study.

Prior to analysis, samples were thawed at 4 ± 1 °C for 24 h. After thawing of the herring blocks, the light and the dark muscles of herring were manually separated. The effect of the physical parameters on the quality of frozen fish were studied by measuring liquid holding capacity (LHC), drip loss, peroxide value (PV), thiobarbituric reactive substances (TBARS), free fatty acids (FFA), lipid content, water content, pH value, disulfide bonds content, available and total SH groups.

Analyses of drip loss was performed on whole herring blocks. FFA, PV, TBARS, water content, lipid content and pH were performed separately on the light and dark muscle of herring. All parameters except drip loss were tested on the light muscle. All chemical analysis were performed at least in triplicate (n = 3).

3.2 Liquid holding capacity (LHC) and drip loss

Liquid holding capacity (LHC) was determined by a centrifugation method. The light muscle was coarsely minced in a Braun Mixer (Type 4262, Germany) for 10-15sec and 2g of the sample was weighed in the glass. Samples were centrifuged at 210×g for 5min at 4°C in tubes fitted with a centrally placed plastic mesh which allow liquid to drain freely from the sample during centrifugation. Centrifugation loss of liquid was calculated as the difference in weight before and after centrifugation. The water content of samples before and after centrifugation were measured to calculate LHC. LHC was calculated as the ratio of remaining liquid compared to the liquid content in the sample before centrifugation. The liquid loss after centrifugation included water, lipids and soluble protein. The ratio of water/oil in the liquid loss was also calculated in this study (Appendix).

Drip loss after frozen storage was determined by comparing the weight of the thawed fillets to the weight of the frozen fillets. Drip loss during thawing was calculated according to the equation:

$$\text{Drip loss (\%)} = \frac{\text{weight of frozen-weight of thawed}}{\text{weight of frozen}} \times 100$$

3.3 Peroxide value (PV)

Lipid hydroperoxide value (PV) was determined with a modified method of ferric thiocyanate (Santha and Decker, 1994). Total lipids were extracted from 5.0 g of samples with 10 mL ice-cold chloroform:methanol (1:1) solution, containing 500 ppm BHT to prevent further peroxidation during the extraction process. Sodium chloride (0.5 M) was added (5.0 mL) into the mixture and homogenized for 10 s (Ultra-Turrax T-25 basic, IKA, Germany) before centrifugation at 5100 rpm for 5 min (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected (500 μL) and mixed with 500 μL ice-cold chloroform: methanol solution. A total amount of 5 μL of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1) was finally added. The samples were brought to room temperature for 10 min and measured at 500 nm on a microplate reader (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as mmol lipid hydroperoxides/kg of wet muscle.

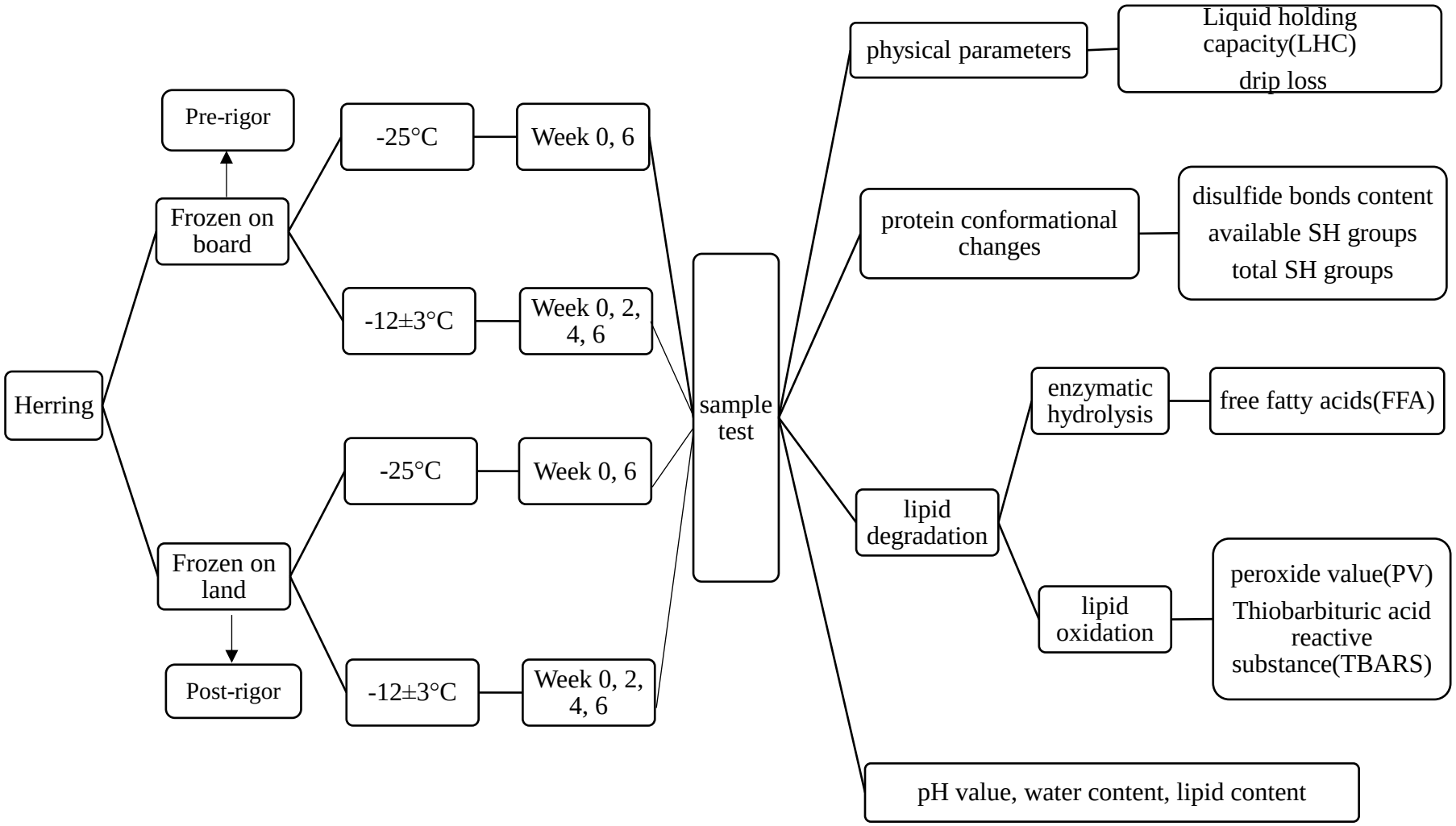

Figure 1: Experimental design diagram of the project

3.4 Thiobarbituric acid reactive substance (TBARS)

A modified method of Lemon (1975) was used to measure thiobarbituric acid reactive substance (TBARS). A fish muscle sample (5.0 g) was homogenized with 10.0 mL of trichloroacetic acid (TCA) extraction solution (mixture of 7.5% TCA, 0.1% propyl gallate and 0.1% EDTA in phosphate buffer) using a homogenizer for 20 s (Ultra-Turrax T-25 basic, IKA, Germany). The homogenized samples were then centrifuged at 5100 rpm for 20 min (TJ-25 Centrifuge, Beckmann Coulter, USA). Supernatant (0.2 mL) was collected and mixed with 0.8mL of 0.02 M thiobarbituric acid and heated in a water bath at 95°C for 40 min. The samples were cooled down on ice and loaded 200μL samples into a 96-well microplate (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for the measurement of absorbance at 530 nm (Tecan Sunrise, Austria). A standard curve was prepared using tetraethoxypropane. The results were expressed as μmol malonaldehyde diethylacetal per kg wet muscle.

3.5 Lipid extraction, lipid content and free fatty acid (FFA)

Total lipid extraction was carried out based on the Bligh and Dyer's method (1959) with adaptations. To a 250 mL centrifuge bottle containing 25 g of sample, 50 mL methanol (MeOH) and 25 mL chloroform ($CHCl_3$) were added and the mixture was homogenized for 2 min. 25 mL $CHCl_3$ was added a second time and the mixture was shaken vigorously for 1 min. 25 mL 0.88% KCl was added and mixed for 1 min. The final mixture was centrifuged for 20 min at 2500 rpm at 4 °C. The total lipid exact (lower layer) was filtrated on a glass microfiber under suction and collected for later analysis. And the lipid content was determined gravimetrically and results was expressed as grams lipid/100 wet muscle.

Free fatty acid (FFA) content was determined on the total lipid extract according to the method of Lowry and Tinsley (1976), with modified method (Bernardez *et al*., 2005). All solvents were evaporated with nitrogen, and 3 mL of cyclohexane was added, followed by 1 mL of cupric acetate-pyridine reagent with agitation to the biphasic system for 40 s. After centrifugation at 2000× g for 10 min, the absorbance of the upper layer was read at 710 nm. The FFA concentration was calculated as μM quantities of oleic acid based on a standard curve in 2–22 μM range. Results were expressed as grams FFA/100 g of lipids.

3.6 Disulfide bond content of protein

The disulfide bond content was measured by using 2-nitro-5-thiosulfobenzoate (NTSB) according to the method of Thannhauser *et al*. (1984). 0.25 mL protein solution was added to 3 mL of prepared NTSB assay solution, pH 9.5. The pH was adjusted with 10% HCl or 10% NaOH solution. The solution was fully mixed. The mixture was incubated in a dark place at room temperature for 25 min. After the reaction, the absorbance of the solution was measured at 412 nm by using a UV-1800 spectrophotometer. The total disulfide content was calculated by using a molar extinction coefficient of 13,900 M^{-1} cm^{-1}.

3.7 Sulfhydryl group content of protein

The sulfhydryl group content was measured by the method of Beveridge *et al*. (1974). Protein samples were extracted by1M NaCl buffer, then the protein extraction was analysed for total sulfhydryl groups and available sulfhydryl groups.

For the total sulfhydryl content, 0.5 mL of the protein solution was added to 2.5 mL of Tris–SDS buffer pH 8.0 (0.1 M Tris, 3 mM EDTA, 0.1 M glycine, 3% sodium dodecyl sulphate-SDS and 8 M urea). To this solution 100 µL of Ellmańs reagent (2 mM 5,5'-dithio-bis(2-nitrobenzoic acid) was added, mixed and incubated in a dark place at room temperature for 30 min. After the reaction, the absorbance of the solution was measured at 412 nm by using the UV spectrophotometer and total SH content was calculated by using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

The available sulfhydryl (SH) content was determined by adding 0.5 ml of protein solution to 2.5 mL of Tris buffer pH 8.0 (0.1 M Tris, 3 mM EDTA, 0.1 M glycine and 8 M urea). Then a procedure for measurement on total SH groups was followed (Beveridge *et al*., 1974).

3.8 Water content

Water content was determined by weight difference of the homogenized muscle samples before and after drying for 4 h at 102-104 °C (ISO, 1993).

3.9 pH measurement

pH was measured by directly inserting the pH detector's probe (Radiometer PHM80 Portable pH meter, Denmark) into the muscle samples.

3.10 Statistical analysis

Statistical analysis of data were carried out by using Microsoft Excel 2013. Means were compared by using ANOVA and Duncan's Multiple-Comparison Test using NCSS 2000 software (NCSS, Kaysville, Utah, USA). Differences between samples were considered to be significant at $p < 0.05$.

4 RESULTS

4.1 pH

The pH changes in relation to the frozen storage time were presented in Figure 2. The pH value of herring increased after 6 weeks storage time ($P < 0.05$) for all the samples. There were significant differences between pH of light muscle and dark muscle ($p<0.05$). Not only for light muscle but also for dark muscle, pH of herring frozen on land was higher than herring frozen on board ($p<0.05$). The pH changes of herring frozen on board and frozen on land had the same trend.

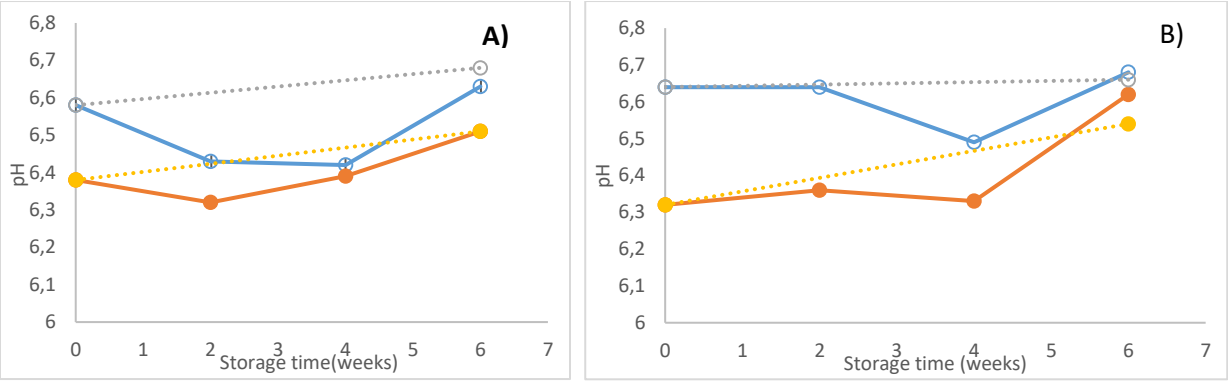

Figure 2: pH changes of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12 °C (solid) and -25 °C (dotted) for up to 6 weeks. Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=3).

The pH of light muscle stored at -12 °C decreased slightly at the beginning storage time, then increased after 2 weeks for land frozen herring and 4 weeks for sea frozen herring (Figure 2A). As shown in Figure 2B, pH of dark muscle stored at -12 °C decreased slightly after 2 weeks, then increased after 4 weeks for both land frozen and sea frozen herring.

Storage temperature had little effect on the pH value. The finial pH of light muscle for 6 weeks at -12 °C had no difference with pH of light muscle at -25 °C ($p>0.05$). The same result can be seen for the dark muscle.

4.2 Water content

The water content of light muscle and dark muscle were very different (Figure 3). At the week 0, the water content of the light muscle of herring frozen on board and frozen on land was similar, ranging from 72.16% to 73.43%, while the water content of the dark muscle of herring frozen on board and frozen on land was ranging from 62.12% to 63.56%. The dark muscle of herring had significantly lower water content than its light part.

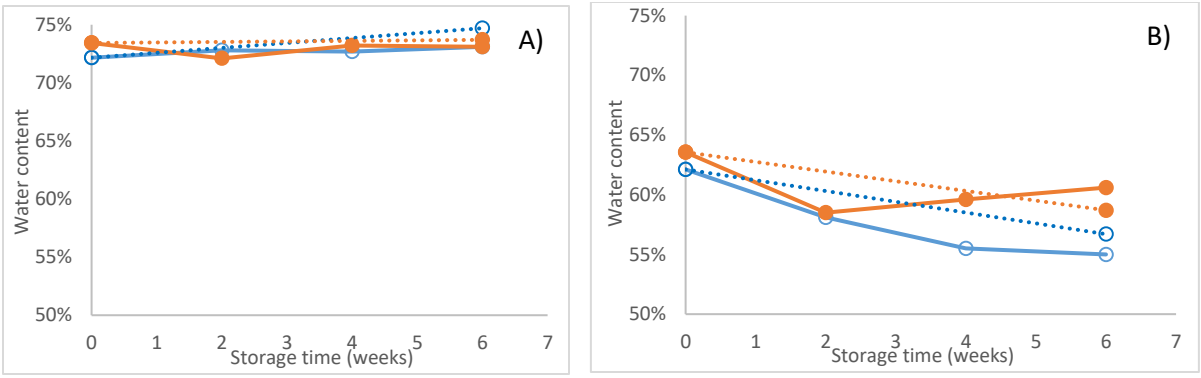

Figure 3: Water content of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks.

As seen in Figure 3A, the water content of the light muscle was rather stable during the storage time for herring frozen on board and on land both at -12 °C and -25 °C.

Obvious decrease of water content of the dark muscle during storage was obtained (Figure 3B). Water content of the dark muscle in herring frozen on board was lower than that of frozen on land and decreased more sharply during storage than the frozen on land. Compared to -12 °C, the sample at -25 °C was more stable in water content ranging from 62.12% to 56.70% of herring frozen on board and 63.56% to 58.70% of herring frozen on land.

4.3 Lipid content

Considerable difference in lipid content was observed between the light and dark muscles ($p<0.05$) (Figure 4). Moreover, the initial lipid content of the light muscle from herring frozen on board was significantly higher than that from frozen on land ($p<0.05$), which is perhaps due to material difference. The lipid content of the light muscle for all the samples trended to decrease with storage time. The lipid content of light muscle ranged from 7.44% to 6.00% for herring frozen on board and from5.98% to 6.08% for herring frozen on land. There was no significant difference between frozen on board and frozen on land during the storage time ($p>0.05$). For the -25 °C samples, the lipid content of frozen on board was 4.94%, which was lower than -12 °C samples ($p<0.05$).

The lipid content of the dark muscle varied considerably, especially the dark muscle from herring frozen on board. The lipid content of the dark muscle from herring frozen on board and stored at -12 °C ranged from 18.61% to 29.06%, while samples stored at -25 °C ranged from 18.61% to 25.81%. The lipid content of dark muscle of frozen on land herring was more stable than in material frozen on board, which varied from18.18% to 21.64% of -12°C sample. There was no significant difference between lipid content of sample at -12°C and -25°C ($p>0.05$).

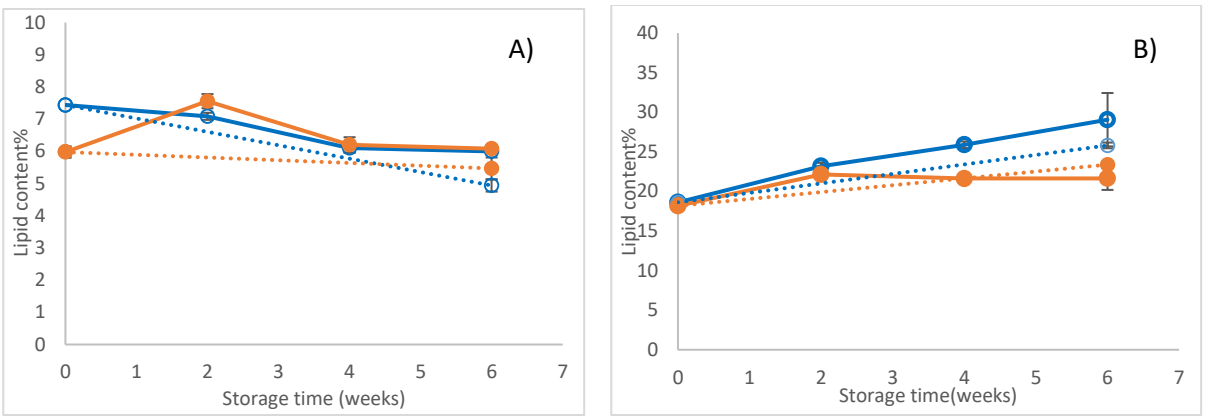

Figure 4: Lipid content of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=6).

4.4 Liquid holding capacity and drip loss

Drip loss was calculated as a percentage of weight loss before and after thawing. As shown in Figure 5, with extended storage time, the trend of drip loss change was increasing for both herring frozen on board and on land. At week 6, the drip loss of herring frozen on land and stored at -12 °C was 5.24%, while drip loss at -25 °C was 4.72% which only increased slightly compared to week 0. Drip loss of herring frozen on land was obviously higher than that of frozen on board. At week 6, the drip loss of herring frozen on board and stored at -12 °C was 3.11%, while drip loss at -25°C was 2.25%. According to the results, herring frozen on board and stored at -25°C had beneficial effects in reducing drip loss.

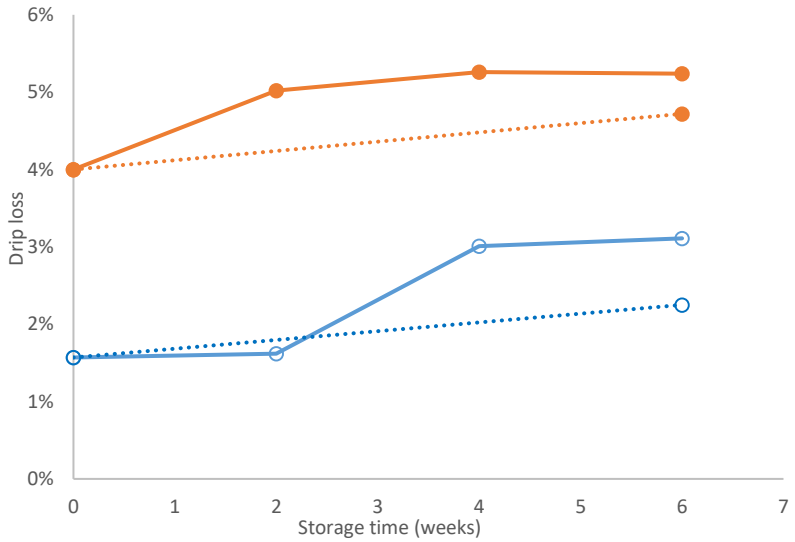

Figure 5: Drip loss of herring frozen on board (○) and frozen on land (●) during frozen storage at -12° C (solid) and -25° C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks.

Usually water holding capacity and drip loss are related to the protein denaturation during frozen storage. For fatty fish, the lost liquid during thawing or centrifugation contained not only water, but also some oil and soluble protein. Therefore, liquid holding capacity was used for fatty fish, which was more reasonable than water holding capacity (Figure 6).

The liquid holding capacity (LHC) of herring frozen on board and stored at -12 °C decreased from 91.61% to 79.50% within 4 weeks of storage, followed by increase to 82.66% at week 6. The LHC of herring frozen on land and stored at -12 °C showed similar trend, decreased from 90.72% to 83.51%, then increased to relatively high 95.18%. Compared with -12 °C samples at week 6, -25 °C samples seemed the same effect on LHC. The LHC for frozen on board and on land was 82.77% and 94.99%, respectively.

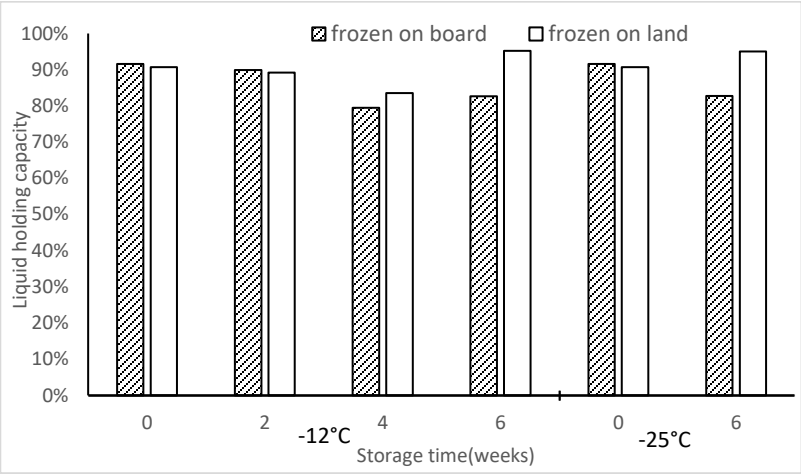

Figure 6: Liquid holding capacity of herring frozen on board and frozen on land during frozen storage at -12°C and -25°C. Fluctuant -12^{o} C sample was stored at fluctuant -12^{o} C for 4 weeks, then at -25^{o} C for additional 2 weeks.

Liquid holding capacity are not only related to protein denaturation, but also enzymatic hydrolysis and oxidation of lipid. In order to know the effect of protein denaturation and enzymatic hydrolysis of lipid to LHC, the ratio of water to oil in the lost liquid during centrifugation was calculated (Figure 7).

The ratio of water/oil of herring both frozen on board and on land and stored at -12 °C was relatively stable, which maintained around 3.2 during 4 weeks. However, the ratio of water/oil for herring frozen on board increased to 4.47 at week 6, which indicates that during centrifugation the muscle lose more water than before and the water holding capacity decreased. For samples stored at -25 °C, the ratio of water/oil of herring frozen on board and on land both increased. The increase was however higher for the frozen on land sample higher than the samples frozen on board.

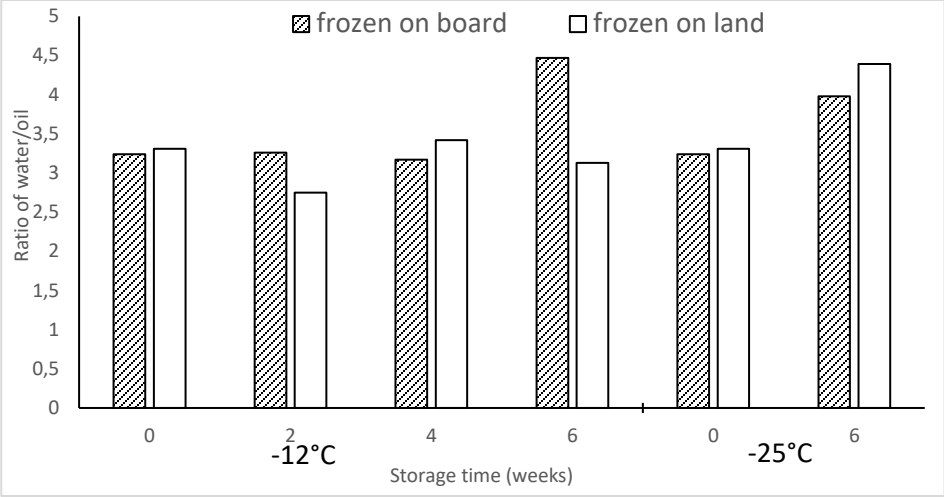

Figure 7: Ratio of water/oil in lost liquid of herring frozen on board and frozen on land during frozen storage at -12°C and -25°C. Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks.

4.5 Peroxide value (PV)

PV was a primary oxidation product during lipid oxidation. A significant difference in peroxide value (PV) was observed between the light muscle and the dark muscle (Figure 8). For the light muscle, PV of herring frozen on board and stored at -12 °C was obviously lower than that of frozen on land ($p<0.05$). For frozen at land, PV increased the first 2 weeks followed by decrease at week 4, and then increased again to 89.36 μmol/kg ($p<0.05$). While PV of frozen at board increased during 6 weeks, then finally reached 50.89 μmol/kg at week 6.

Compared with -12 °C, the PV at -25 °C were more stable and maintained a low level. There was no significant difference between week 0 and week 6 for samples stored at -25 °C for frozen at board sample ($p>0.05$). While for frozen at land sample, PV decreased to 18.77 μmol/kg ($p<0.05$). The results showed that lower storage temperature had strong effect on inhibiting the lipid oxidation process.

For the dark muscle, the opposite trend to the light muscle was observed, where the PV of herring frozen on board was higher than that of frozen on land ($p<0.05$). The trend of PV changes for frozen on board and on land were the same. The PV of samples stored at -12 °C increased during 4 weeks, then sharply declined to a low level ($p<0.05$) at week 6. For both herring frozen on board and land and stored at -25 °C, the PV decreased significantly at week 6 ($p<0.05$).

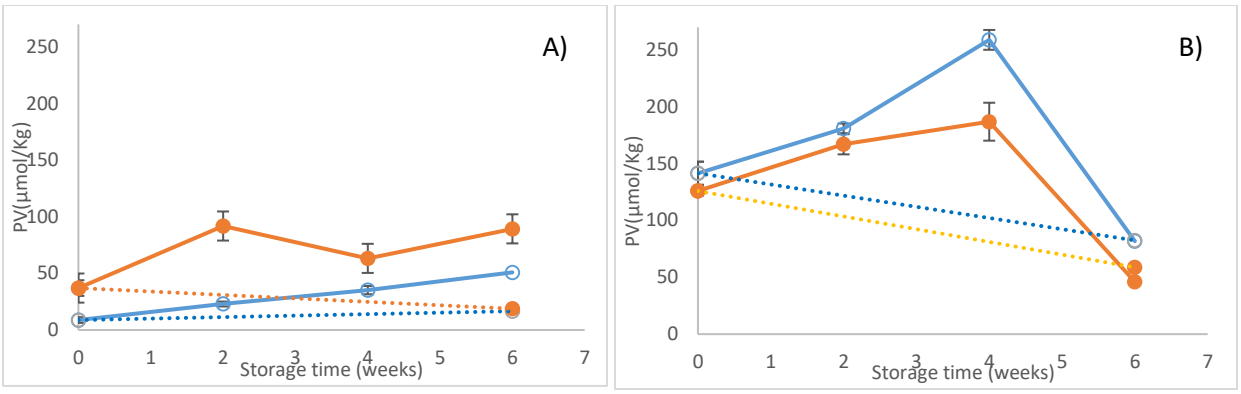

Figure 8: PV of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=6).

4.6 Thiobarbituric reactive substances (TBARS)

There were significant differences in TBARS between the light muscle and the dark muscle (Figure 9). For the light muscle and dark muscle, TBARS of herring frozen on board and stored at -12 °C was obviously lower than that of frozen on land ($p<0.05$). Light muscle and dark muscle showed similar trend in formation and decomposition of TBARS. The TBARS of samples stored at -12 °C increased the first 2 weeks and decreased at 4 weeks, then increased again. For samples at -25 °C, the TBARS of all samples obviously increased ($p<0.05$). There were significant differences between -25°C samples and -12°C samples at week 6 ($p<0.05$), except dark muscle of frozen on land. The results showed that storage temperature had some effect on the lipid oxidation process.

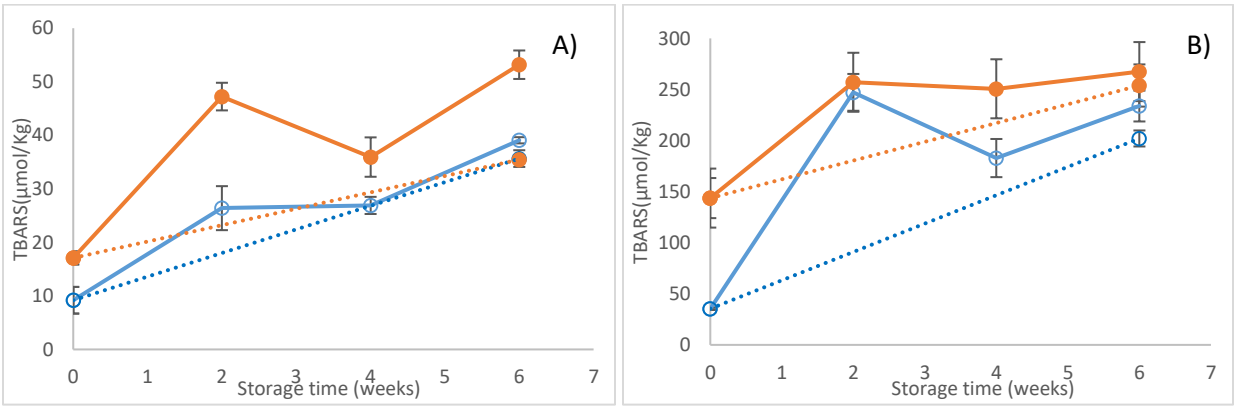

Figure 9: TBARS of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=6).

4.7 Free fatty acids (FFA)

Lipid hydrolysis occurred in the light muscle and dark muscle during storage time. The light muscle of all the samples had generally higher free fatty acid (FFA) content than the dark muscle, except at week 0 (Figure 10). The effect of storage temperature was evident where the FFAs of all samples stored at -25°C were much lower than FFAs of -12 °C samples ($p<0.05$). It suggested that lower storage temperature showed partial inhibition of lipid hydrolysis compared with higher temperature.

For the light muscle, FFA content of samples stored at -12 °C increased during 4 weeks, then decreased slightly at week 6. There were no significant differences between frozen on board and frozen on land when at -12°C ($p>0.05$), except week 6 ($p<0.05$). For the dark muscle, the FFA in samples stored at -12 °C increased first, followed by decrease. Samples stored at -25°C showed also showed decreased trend in FFA and frozen on board and on land had no difference at week 6 ($p>0.05$).

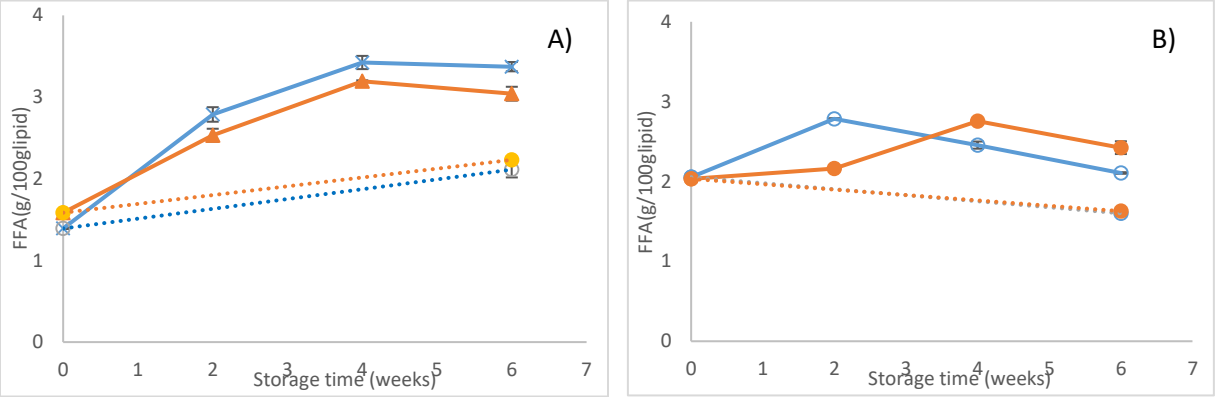

Figure 10: FFA of A) light muscle and B) dark muscle of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=6).

4.8 Disulfide bond content of protein

The changes in disulfide bond content of the protein are shown in Figure 11. The formation of disulfide bonds content of both frozen on board and frozen on land material increased significantly with extended storage time ($p<0.05$). The disulfide bond content of herring frozen on land and stored at -12°C was significantly higher than that of herring frozen on board ($p<0.05$) before week 4. However, there is no difference between samples stored at -12 °C and -25°C at week 6 ($p>0.05$).

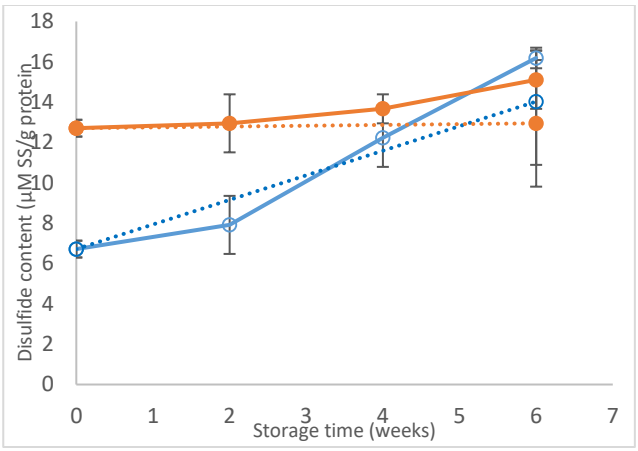

Figure 11: Disulfide bond content of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=3).

4.9 Sulfhydryl group content of protein

Total sulfhydryl content of herring frozen on board and frozen on land showed the same trend (Figure 12), decreasing during frozen storage time. At week 6, the total sulfhydryl content of herring frozen on board and frozen on land and stored at -12 °C decreased by 27.30% and 30.76%, respectively, compared to week 0. There was no significant difference between herring frozen on board and frozen on land ($p>0.05$). It may be due to a short frozen storage time. For -25 °C sample, total sulfhydryl content of herring frozen on board and frozen on land at week 6 decreased by 21.81% and 13.97%, respectively, compared to week 0. But there were no significant differences between -12 °C samples and -25 °C samples at week 6 ($p>0.05$).

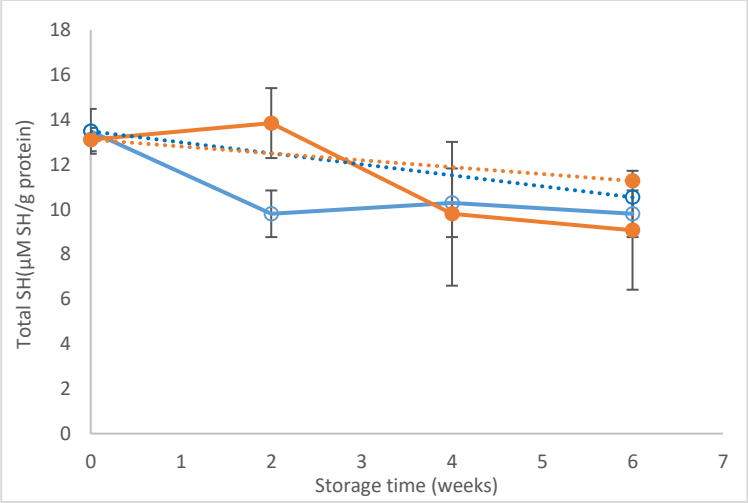

Figure 12: Total sulfhydryl content of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=3).

Available sulfhydryl content of herring frozen on board and frozen on land had the same trend (Figure 13). In generally, they increased during frozen storage time. At week 6, available sulfhydryl content of herring frozen on board and frozen on land at -12°C increased by 56.29% and 41.12%, respectively. The available SH groups of herring frozen on land were higher than those of herring frozen on board, but there was no significant difference between them ($p>0.05$). For -25°C sample, available sulfhydryl content of herring frozen on board and frozen on land at week 6 increased by 56.29% and 47.04%, respectively, compared to week 0. But there were no significant differences between -12°C samples and -25°C samples at week 6 ($p>0.05$).

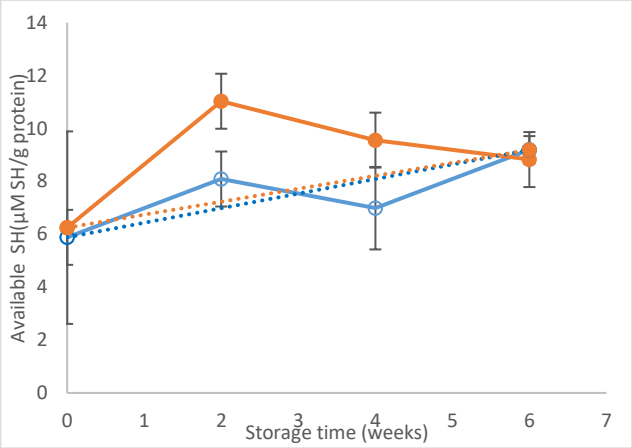

Figure 13: Available sulfhydryl content of herring frozen on board (○) and frozen on land (●) during frozen storage at -12°C (solid) and -25°C (dotted). Fluctuant -12° C sample was stored at fluctuant -12° C for 4 weeks, then at -25° C for additional 2 weeks. Bars represents standard deviation for the samples tested (n=3).

5 DISCUSSION

5.1 pH

Usually pH decreases due to anaerobic formation of lactic acids during the first hours after death. During later post mortem changes, pH increases slightly because of the formation of alkaline compounds (Huss, 1988). In our experiment, the pH value decreased initially and then increased. Similar observations were made by other researchers (Alasalvar et al., 2001; Manju et al., 2007). The data revealed that variations in pH of herring frozen on board have the similar trend with those of herring frozen on land, but pH of light muscle from frozen on board at -12^{o} C increased later than that from frozen on land during storage time.

The results showed that pH of herring frozen on board was higher than that of frozen on land and the light muscle pH of frozen on board increased two weeks later than that of frozen on land. This maybe because that the frozen on board fish was frozen at the pre-rigor time. So freezing could slow down the rate of pH decline, then prolong the time for decreasing. The pH value was higher and decreasing period lasted longer time than post-rigor frozen herring.

5.2 Lipid content

Although there was only a little change, the decrease trend of lipid content in light muscle could be observed. This was related to lipid degradation during the storage time, which can cause lipid inside the cell set free. So the lipid content in the muscle decreased. This also indicates the liquid holding capacity and drip loss will be changed. In the results, lipid content of dark muscle from herring frozen on board increased even higher than that from frozen on land. This maybe because the water content of it decreased significantly ($p<0.05$).

5.3 LHC and drip loss

Physical properties of fish muscle such as liquid holding capacity and drip loss are highly related to the change of microstructure of fish muscle. These properties are highly depended on the conditions of myofibrillar protein and lipid oxidation process. Depolymerisation of myofibrillar proteins results in the formation of a homogeneous protein matrix, where pores and gaps appear in the protein matrix, thus enhancing liquid loss (Ofstad *et al*., 1995).

The LHC and drip loss of herring frozen on board and frozen on land were obviously affected during the frozen storage. Also the storage temperature showed effect on these physical properties. The results of liquid holding capacity were not easily analyzed, which was perhaps due to the raw material fluctuant reason and sampling method. For herring frozen on board, there is one pumping time to take fish into the hall. While for herring frozen on land, there are two pumping times. So the chance to get gaping and collision for herring frozen on land is higher than herring frozen on land. The frozen on land should had negative effect on liquid holding capacity. Moreover, it was observed that a low pH was associated with reduced water holding capacity (Ofstad *et al*., 1996). Previously the pH of herring frozen on land is lower than that of frozen on board. This also showed the same results. From the results of drip loss, it was very clear that -25°C showed less deterioration effects compared with fluctuant -12°C and frozen on board sample had better effect on these physical properties than frozen on land.

5.4 Lipid degradation

In order to investigate the progress of lipid degradation, lipid oxidation product peroxide value (primary product), TBARS (secondary products) and free fatty acids were measured. From the results of PV, TBARS and FFA, it proved that storage time and temperature very important to lipid degradation of herring dark and light muscle. And -25°C showed more preservative effects compared to fluctuant -12°C. Obvious difference was observed between light and dark muscle of all samples where the dark muscle was more prone to lipid oxidation throughout the storage time which is the same with other studies (Undeland *et al*., 1998)

For the light muscle, PV of herring frozen on board at -12°C was obviously lower than that of frozen on land ($p<0.05$). It was because herring frozen on land is usually stored at -1.5°C for 2-3 days, while herring frozen on board is only stored for 1-5 hours. So the texture of herring frozen on land has longer time open to the air and then oxidation happens. For dark muscle, the opposite trend to light muscle, PV of herring frozen on board was higher than that of frozen on land ($p<0.05$). Herring frozen on board is frozen during pre-rigor period, then there is more oxygen in blood stored in dark muscle. Herring are frozen as big blocks and oxidation continue inside the block. While herring frozen on land has enough time to release the oxygen from the dark muscle when it is stored at -1.5 ºC for 2-3 days.

PV at -12°C increased during 4 weeks, then sharply declined to a low level ($p<0.05$) at week 6. For both herring, PV at -25°C decreased significantly ($p<0.05$) at week 6. The decrease of PV during storage time suggested that hydroperoxide formed might be decomposed to other oxidative compounds (Frankel, 1998). Decrease in TBARS also could be seen in all the sample at -12°C. The decreased in TBARS might be related to a loss of low molecular weight decomposition products during the advancement of oxidation (Nawar, 1996).

The FFA of light muscle for all the samples were generally higher than dark muscle. This may be due to higher enzymatic activity in light muscle and FFAs as a product of enzymatic hydrolysis are produced during frozen storage (Losada *et al*., 2007). Enzymatic activity is proportionally higher in samples with the lower lipid content. Karlsdottir *et al*. (2014) found that higher FFA content was associated with lower lipid content. As described before, the lipid content of light muscle decreased, while lipid content of dark muscle increased. According to Figure 10, the trend of FFA of light muscle increased and FFA of dark muscle decreased. Our results were consistent with this finding.

5.5 Protein conformation changes

The decrease in total sulfhydryl content was due to sulfhydryl oxidation of myofibrillar proteins. During the frozen storage, exposure of reactive sulfhydryl groups were prone to oxidation or disulfide interchange. For herring frozen on board and frozen on land, decrease in total sulfhydryl groups was found during the storage time at -12°C and -25°C. This was presumed that formaldehyde formed during storage induced the aggregation of protein, which caused the oxidation of sulfhydryl groups.

While increase in available SH groups were found in the results. For both frozen herring, available SH groups increased a little during the frozen storage at -12°C and -25°C.The increase in available SH groups may be due to the conformational changes of protein during frozen storage, which lead to exposure of masked SH groups. Since the SH groups of protein are exposed, more disulfide

bonds are formed due to the increase in interaction of the interior and exterior amino acids (Hsu *et al*., 2007; Ko *et al*., 2007; Raikos *et al*., 2007), thereby decreasing the total SH groups. Furthermore, it is possibly related to enzyme activity (Stoknes *et al*., 2005) that further degrades the proteins, resulting in additional exposure of SH groups.

The decrease in sulfhydryl groups with a concomitant disulfide bond formation could be seen in the study. This may be due to either the oxidation of sulfhydryl groups, disulfide interchanges or the formation of hydrogen and hydrophobic bonds (Hsu *et al*., 2007; Ko *et al*., 2007; Raikos *et al*., 2007; Sannaveerappa *et al*., 2004). Obvious increase in disulfide bond was observed in this study. After 6 weeks of storage, disulfide bond in herring frozen on board and frozen on land at -12C increased by 141.28% and 18.88%, respectively. The increased disulfide bond formation might due to the accelerated denaturation of myosin molecules, especially the conformational changes, in which the reactive sulfhydryl groups were exposed to oxidation.

The total SH groups and available SH groups of herring frozen on land seemed higher than those of herring frozen on board, but there were no significant differences between them ($p>0.05$). And also for -12°C and -25°C, no significant differences had been seen in the results. This may be because the frozen storage time was so short and the conformational changes of protein was not so obviously. And also some steps during the measurement should be improved to reduce the deviation of the results.

6 CONCLUSION

The results of this study indicate that both frozen handling method and storage temperature significantly affects the physicochemical properties of herring during frozen storage. Compared the storage temperature, -25°C proved to have more preservative effects than fluctuant -12°C. It had significant positive effects on maintaining water content and drip loss, reducing the lipid degradation including PV, TBARS and FFA content.

Compared the different handling methods of herring, frozen on board had more advantages than frozen on land although they had no significant differences in some parameters. Herring frozen on board was frozen in pre-rigor state, so the pH change of it was more moderate than herring frozen on land. And frozen on board has less drip loss, PV and TBARS compared with frozen on land, but it seemed no effects on lipid enzymatic hydrolysis (FFA). Moreover, the disulfide content of herring frozen on board was lower than that of frozen on land.

In conclusion, storage temperature should be controlled strictly during the fatty fish logistics and transportation. Low temperature -25°C can effectively reduce drip loss, especially inhibit the lipid degradation of fatty fish. Freezing herring during pre-rigor period can slow down lipid oxidation and reduce the protein denaturation caused by freezing process.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Magnea G. Karlsdottir and Sigurjon Arason for their elaborate guidance, valuable advice and help to me.

I am deeply grateful to Tumi Tomasson, Thor Asgeirsson, Sigridur Kr. Ingvarsdottir and Mary Frances Davidson for giving me the opportunity to participate in the programme and their continued assistance, support and guidance during the six month period of the training programme. Also thanks to for their careful care to my life here.

Special thanks to Paulina, Huong, Cyprian and Svanhildur for their enthusiastic guidance and assistance in chemical analysis. Thanks to Matis to provide me the platform for experiments.

At last I would like to thank all the UNU-FTP fellows, especially Quality Management of Fish Handling and Processing's fellows, for their help and friendship during my study and live in Iceland.

LIST OF REFERENCES

Alasalvar, C., Taylor, K. D., Oksuz, A., Garthwaite, T., Alexis, M. N., & Grigorakis.K. (2001). Freshness assessment of cultured seabream (Sparus aurata) by chemical, physical, and sensory methods. *Food Chemistry, 72*, 33-40.

Aubourg, S. P., Quitral, V., & Larrain, M. A. (2007). Autolytic degradation and microbiological activity in farmed Coho salmon (Oncorhynchus kisutch) during chilled storage. *Food Chemistry, 104*(1), 369–375.

Azam, K., Mackie, I. M., & Smith, J. (1989). The effect of slaughter method on the quality of rainbow trout (Salmo salar) during ice storage. *International Journal of Food Science and Technology, 24*, 69-79.

Badii , F., & Howell, N. (2002a). Changes in the texture and structure of cod and haddock fillets during frozen storage. *Food Hydrocolloids, 16*(4), 313-319.

Badii, F., & Howell, N. (2002b). A comparison of biochemical changes in cod (Gadus morhua) and haddock (Melanogrammus aeglefinus) fillets during frozen storage. *Journal of the Science of Food and Agriculture, 82*(1), 87-97.

Bak, L., Andersen, A., Andersen, E., & Bertelsen, G. (1999). Effect of modified atmosphere packaging on oxidative changes in frozen stored cold water shrimp (Pandalus borealis). *Food Chemistry, 64*(2), 169–175.

Ben-gigirey, B., De Sousa, J., Villa, T., & Barros-velazquez, J. (1999). Chemical Changes and Visual Appearance of Albacore Tuna as Related to Frozen Storage. *Journal of Food Science, 64*(1), 20-24.

Bernardez, M., Pastoriza, L., Sampedro, G., Herrera, J. J., & Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agriculture and Food Chemistry, 53*, 1903–1906.

Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science, 39*, 49-51.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911-917.

Boran, G., Karacam, H., & Boran, M. (2006). Changes in the quality of fish oil due to storage temperature and time. *Food Chemistry, 98*, 693-698.

Careche, M., Mazo, M., Torrejon, P., & Tejada, M. (1998). Importance of frozen storage temperature in the type of aggregation of myofibrillar proteins in cod (Gadus morhua) fillets. *Journal of Agricultural and Food Chemistry, 46*, 1539-1546.

Carton, I., Bocker, U., Ofstad, R., & Sørheim, O. (2009). Monitoring secondary structural changes in salted and smoked salmon muscle myofiber proteins by FT-IR microspectroscopy. *Journal of Agricultural and Food Chemistry, 57*(9), 3563–3570.

Dawood, A. A., Roy, R. N., & Williams, C. S. (1986). Effects of delayed icing on the storage life of rainbow trout. *Journal of Food Technology, 21*(2), 15-166.

Erikson, U. (2000). *Rigor measurements.* (K. C. S, & W. D. P, Eds.) Cornwall, UK: Fishing News Books.

FAO. (2014). *The Herring.* [February 2015] <www.fao.org/wairdocs/tan/x5933e/x5933e01.htm>

Frankel, N. (1998). Methods to determine extent of oxidation. In N. Frankel, *Lipid oxidation* (pp. 79-98). Dundee, Scotland: Oily Press.

Gormley, R., Walshe, T., Hussey, K., & Butler, F. (2002). The Effect of Fluctuating vs. Constant Frozen Storage Temperature Regimes on Some Quality Parameters of Selected Food Products. *LWT - Food Science and Technology, 35*(2), 190-200.

Haard, N. (1992). *Biochemical reactions in fish muscle during frozen storage. Ch. 20 in Seafood Science and Technology, E.G. Bligh (Ed.).* Oxford: Fishing News Books.

Hagyard, C., Keiller, A., & Cu, T. (1993). Frozen storage conditions and rancid flavour development in lamb. *Meat Science, 35*(3), 305–312.

Hattula, T., Luoma, T., Kostiainen, R., Poutanen, J., Kallio, M., & Suuronen, P. (1995). Effects of catching method on different quality parameters of Baltic herring (Clupea harengus L.). *Fisheries Research, 23*(4), 209-221.

Hsu, K. C., Hwang, J. S., Yu, C. C., & Jao, C. L. (2007). Changes in conformation and in sulfhydryl groups of actomyosin of tilapia (Orechromis niloticus) on hydrostatic pressure treatment. *Food Chemistry, 103*, 560-564.

Huss, H. H. (1988). *Fresh fish- quality and quality changes.* Training Manual Rome: Food and Agriculture Organization and Denish International Development Agency.

Icelandic Ministry of Fisheries.. *Atlantic mackerel.* [January 2014] <http://www.fisheries.is/main-species/pelagic-fishes/atlantic-mackerel/>

ISO. (1993). *Determination of moisture and other volatile matter content.* Geneva, Switzerland: The International Organization for Standardization.

Jerrett, A. R., Holland, A. J., & Cleaver, S. E. (1998). Rigor contractions in rested and partially exercised chinook salmon white muscle as affected by temperature. *Journal of Food Science, 63*(1), 53-56.

Karlsdottir, M. G., Sveinsdottir, K., Kristinsson, H. G., Villot, D., Craft, B. D., & Arason, S. (2014). Effects of temperature during frozen storage on lipid deterioration of saithe (Pollachius virens) and hoki (Macruronus novaezelandiae) muscles. *Food Chemistry, 156*, 234-242.

Keller, M. (2006). *Optimization of Containers for Seaway Logistics of Chilled and Frozen Fishery Products.* Reykjavik: Icelandic Fisheries Laboratories & University of Iceland.

Kiessling, A., Stien, L. H., Torslett, Ø., Suontama, J., & Slinde, E. (2006). Effect of pre- and post-mortem temperature on rigor in Atlantic salmon muscle as measured by four different techniques. *Aquaculture, 259*, 390-402.

Ko, W. C., Yu, C. C., & Hsu, K. C. (2007). Changes in conformation and sulfhydryl groups of tilapia actomyosin by thermal treatment. *LWT-Food Science and Technology, 40*, 1316-1320.

Lauritzsen, K., Martinsen, G., & Olsen, R. L. (1999). Copper induced lipid oxidation during salting of cod (Gadus morhua L.). *Journal of Food Lipids, 6*(4), 299–315.

LeBlanc, E., LeBlanc, R., & Blum, I. (1988). Prediction of quality in frozen cod(Gadus morhua) fillets. *Journal of Food Science, 53*, 328-340.

Lehmann, I., & Aubourg, S. P. (2008). Effect of previous gutting on rancidity development in horse mackerel (Trachurus trachurus) during frozen storage at -20 °C. *International Journal of Food Science & Technology, 43*(2), 270–275.

Lemon, D. W. (1975). An improved TBA test for rancidity. *New Series Circular No. 51, Halifax Laboratory, Halifax, Nova Scotia*

Losada, V., Barros-Velázquez, J., & Aubourg, S. P. (2007). Rancidity development in frozen pelagic fish: influence of slurry ice as preliminary chilling treatment. *LWT-Food Science and Technology, 40*, 991-999.

Love, R. M. (1962). Protein denaturation in frozen fish. VII.—Effect of the onset and resolution of rigor mortis on denaturation. *Journal of the Science of Food and Agriculture, 13*(10), 534-545.

Lowry, R., & Tinsley, I. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society, 53*, 470-472.

Mackie, I. (1993). The effects of freezing on flesh proteins. *Food Reviews International, 9*(4), 575-610.

Mai, N. (2010). *Enhancing quanlity management of fresh fish supply chains through improved logistics and ensured traceability.* Reykjavik: University of Iceland.

Manju, S., Jose, L., Srinivasa Gopal, T. K., Ravishankar, C. N., & Lalitha, K. V. (2007). Effects of sodium acetate dip treatment and vacuum-packaging on chemical, mirobiological, textural and sensory changes of pearlspot (Etroplus suratensis) during chill storage. *Food Chemistry, 102*, 27-35.

Mao, W., & Sterling, C. (1970). Parameters of texture changes in processed fish: cross-linkage of proteins. *Journal of Texture Studies, 1*(4), 484-490.

Martinsdóttir, E., & Magnússon, H. (2001). Keeping Quality of Sea-Frozen Thawed Cod Fillets on Ice. *Journal of food science, 66*(9), 1402-1408.

Moureh, J., & Derens, E. (2000). Numerical modelling of the temperature increase in frozen food packaged in pallets in the distribution chain. *International Journal of Refrigeration, 23*(7), 540–552.

Moureh, J., Laguerre, O., Flick, D., & Commere, B. (2002). Analysis of use of insulating pallet covers for shipping heat-sensitive foodstuffs in ambient conditions. *Computers and Electronics in Agriculture, 34*(3), 89-109.

Nawar, W. (1996). Lipids. In O. R. Fennema, *Food Chemistry* (pp. 225-319). New York: Marcel Dekker.

Nielsen, M., & Jørgensen, B. (2004). Quantitative Relationship between Trimethylamine Oxide Aldolase Activity and Formaldehyde Accumulation in White Muscle from Gadiform Fish during Frozen Storage. *Journal of Agricultural and Food Chemistry, 52*(12), 3814–3822.

Ofstad, R., Kidman, S., Myklebust, R., Olsen, R. L., & Hermansson, A. M. (1995). Liquid holding capacity and structural changes in comminuted salmon (Salmo salar) muscle as influenced by pH, salt and temperature. *LWT - Food Science and Technology, 28*(3), 329-339.

Ofstad, R., Egelandsdal, B., Kidman, S., Myklebust, R., Olsen, R. L., & Hermansson, A. M. (1996). Liquid loss as effected by post mortem ultralstructural changes in fish muscle: cod (Gadus morhua L) and salmon (Salmo salar). *J Sci Food Agric, 71*, 301-312.

Ólafsdóttir, G. (2005). *Volatile compounds as indicators in fish during chilled storage: Evaluation of microbial metabolites by an elctronic nose.* Reykjavik: University of Iceland.

Paredi, M. E., RoldÁn, H. A., & Crupkin, M. (2006). Changes in myofibrillar proteins and lipids of squid (Illex argentinus) during frozen storage. *Journal of Food Biochemistry, 30*(5), 604-621.

Piggott, G., & Tucker, B. (1990). *Effects of technology on nutrition.* New York: Marcel Dekker.

Raab, V., Bruckner, S., Beierle, E., Kampmann, Y., Petersen, B., & Kreyenschmidt, J. (2008). Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. *Journal on chain and network science, 8*(1), 59-73.

Raikos, V., Campell, L., & Euston, S. R. (2007). Effects of sucrose and sodium chloride on foaming properties of egg white proteins. *Food Research International, 40*, 347-355.

Rediers, H., Claes, M., Peeters, L., & Willems, K. (2009). Evaluation of the cold chain of fresh-cut endive from farmer to plate. *Postharvest Biology and Technology, 51*(2), 257-262.

Refsgaard, H. H., Brockhoff, P. M., & Jensen, B. (2000). Free Polyunsaturated Fatty Acids Cause Taste Deterioration of Salmon during Frozen Storage. *Journal of Agricultural and Food Chemistry, 48*(8), 3280–3285.

Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Journal of Food Science, 34*, 441-447.

Saeed, S., & Howell, N. K. (1999). High-performance liquid chromatography and spectroscopic studies on fish oil oxidation products extracted from frozen Atlantic mackerel. *Journal of the American Oil Chemists' Society, 76*(3), 391-397.

Saeed, S., & Howell, N. K. (2001). 12-Lipoxygenase activity in the muscle tissue of Atlantic mackerel (Scomber scombrus) and its prevention by antioxidants. *Journal of the Science of Food and Agriculture, 81*(8), 745–750.

Saeed, S., & Howell, N. (2002). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (Scomber scombrus). Volume 82, Issue 5, pages. *Journal of Science Food Agriculture., 82*(5), 579–586.

Sannaveerappa, T., Ammu, K., & Joseph, J. (2004). Protein-related changes during salting of milkfish (Chanos chanos). *Journal of Science and Food Agriculture, 84*, 863-869.

Santha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Association of Official Analytical Chemists International, 77*, 421–424.

Shenouda, S. Y. (1980). Theories of protein denaturation during frozen storage of fish flesh. In C. O. Chichester, *Advances in Food Research* (pp. 275-311). London: Academic Press.

Sikorski, Z. E., Olley, J., Kostuch, S., & Olcott, H. S. (1976). Protein changes in frozen fish. *C R C Critical Reviews in Food Science and Nutrition, 8*(1), 97-129.

Skjervold, P. O., Fjæra, S. O., Østby, P. B., & Einen, O. (2001a). Livechilling and crowding stress before slaughter of Atlantic salmon (Salmo salar). *Aquaculture, 192*, 2-4.

Skjervold, P. O., Rørå, A. M., Fjæra, S. O., Vegusdal, A., Vorre, A., & Einen, O. (2001b). Effects of pre-, in-, or post-rigor filleting of live chilled Atlantic salmon. *Aquaculture , 194*, 315-326.

Sohn, J., & Ohshima , T. (2010). Lipid oxidation, odour and colour of fish flesh. In C. Alasalvar, F. Shahidi, K. Miyashita, & U. Wan, *Handbook of seafood Quality, Safety and Health Applications* (p. 542). UK: Wiley-Blackwell.

Stien, L. H., Suontama, J., & Kiessling, A. (2006). Image analysis as a tool to quantify rigor contraction in pre-rigor-filleted fillets. *Computers and Electronics in Agriculture, 50*, 109-120.

Stodolnik, I., Stawicka, A., Szczepanik, G., & Aubourg, S. P. (2005). Rancidity inhibition study in frozen whole mackerel (Scomber scombrus) following flaxseed (Linum usitatissimum) extract treatment. *Grasas y aceites, 56*(3), 198-204.

Stoknes, I. S., Walde, P. M., & Synnes, M. (2005). Proteolytic activity in cod (Gadus morhua) muscle during salt curing., 38(6), 693–699. *Food Research International, 38*(6), 693-699.

Thannhauser, T. W., Konishi, Y., & Scheraga, H. A. (1984). Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Analytical Biochemistry*, 181-188.

Tornberg, E., Walgren, M., Brøndum, J., & Engelsen, S. B. (2000). Prerigor conditions in beef under varying temperature and pH falls studied with rigormeter NMR and NIR. *Food Chemistry, 69*, 407-418.

Undeland, I., Stading, M., & Lingnert, H. (1998). Influence of skinning on lipid oxidation in different horizontal layers of herring (Clupea harengus) during frozen storage. *Journal of the Science of Food and Agriculture, 78*, 441-450.

Undeland, I., & Lingnert, H. (1999). Lipid oxidation in fillets of herring (Clupea harengus) during frozen storage. Influence of prefreezing storage. *Journal of agricultural and food chemistry, 47*(5), 2075–2081.

Villemure, G., Simard, R. E., & Picard, G. (1986). Bulk storage of cod fillets and gutted cod (Gadus morhua) under carbon dioxide atmosphere. *Journal of Food Science, 52*(2), 317-320.

Visschers , R., & Jongh, H. (2005). Disulphide bond formation in food protein aggregation and gelation. *Biotechnology Advances, 23*(1), 75-80.

Zhou, A., Lin, L., Liang, Y., Benjakul, S., Shi, X., & Liu, X. (2014). Physicochemical properties of natural actomyosin from threadfin bream (Nemipterus spp.) induced by high hydrostatic pressure. *Food Chemistry, 156*, 402-407.

APPENDIX

The method to calculate the liquid holding capacity (LHC) and ratio of water/oil.

m1: sample weight before centrifugation

m2: sample weight after centrifugation

w1: water content of sample before centrifugation

w2: water content of sample after centrifugation

Δm: liquid loss during centrifugation

The calculation is as following:

Δm=m1-m2

$$\text{Liquid content} = \frac{m1-m2\times(1-w2)}{m1}$$

$$\text{LHC} = 1-\frac{\Delta m}{\text{liquid content}\times m1} = 1-\frac{m1-m2}{m1-m2\times(1-w2)}$$

$$\text{Ratio of water/oil} = \frac{m1\times w1-m2\times w2}{\Delta m-(m1\times w1-m2\times w2)} = \frac{m1\times w1-m2\times w2}{m1-m2-m1\times w1+m2\times w2}$$